

Rapid Communication

An established population of African clawed frogs, *Xenopus laevis* (Daudin, 1802), in mainland ChinaSupen Wang¹, Yufeng Hong² and John Measey^{3,*}¹Chinese Academy of Sciences Key Laboratory of Animal Ecology and Conservation Biology, Institute of Zoology, Beijing, China²Faculty of Land Resource Engineering, Kunming University of Science and Technology, Kunming, China³Centre for Invasion Biology, Department of Botany & Zoology, Stellenbosch University, South AfricaAuthor e-mails: wangsupen@ioz.ac.cn (WS), 5952525@qq.com (YH), john@measey.com (JM)

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Abstract

Reports of amphibian invasions are increasing, although it seems likely that there are more extant populations of alien species than we are currently aware of, and we are far from understanding their full environmental and economic impact. Here we provide data on another established population of African clawed frogs, *Xenopus laevis* (Daudin, 1802); from Yunnan Province in mainland China. The site is an aquaculture area immediately adjacent to the northern shores of Lake Kunming. This report is significant as it is the first known alien population of an albino form of this species, the form that is most prevalent in the pet trade. We call for urgent surveys using eDNA to determine the extent of the invasion of this cryptic amphibian invader around Lake Kunming, as well as studies to determine the environmental and economic impacts at this site, which is already known for an invasion of American bullfrogs, *Lithobates catesbeianus* (Shaw, 1802).

Key words: Anura, *Batrachochytrium dendrobatidis*, Chytridiomycosis, East Asia, Kunming, Pipidae

Introduction

Populations of invasive amphibians have shown a steady increase over time (Kraus 2009), and these populations have brought with them a myriad of environmental and economic impacts that are equivalent to those of freshwater fish and birds (Kraus 2015; Measey et al. 2016). Of those that have Massive or Major impacts (*sensu* Blackburn et al. 2014), three anurans are particularly noteworthy as their invasions are at a multicontinental level: cane toads *Rhinella marina* (Linnaeus, 1758), American bullfrogs *Lithobates catesbeianus* (Shaw, 1802), and African clawed frogs *Xenopus laevis* (Daudin, 1802). Each has had a different initial pathway that has led to these invasions on four or more continents. Cane toads were moved around mostly as biocontrol (Easteal 1981), American bullfrogs were initially moved for aquaculture and then as pets (Kraus 2009), and African clawed frogs firstly for pregnancy testing, later as the model amphibian for

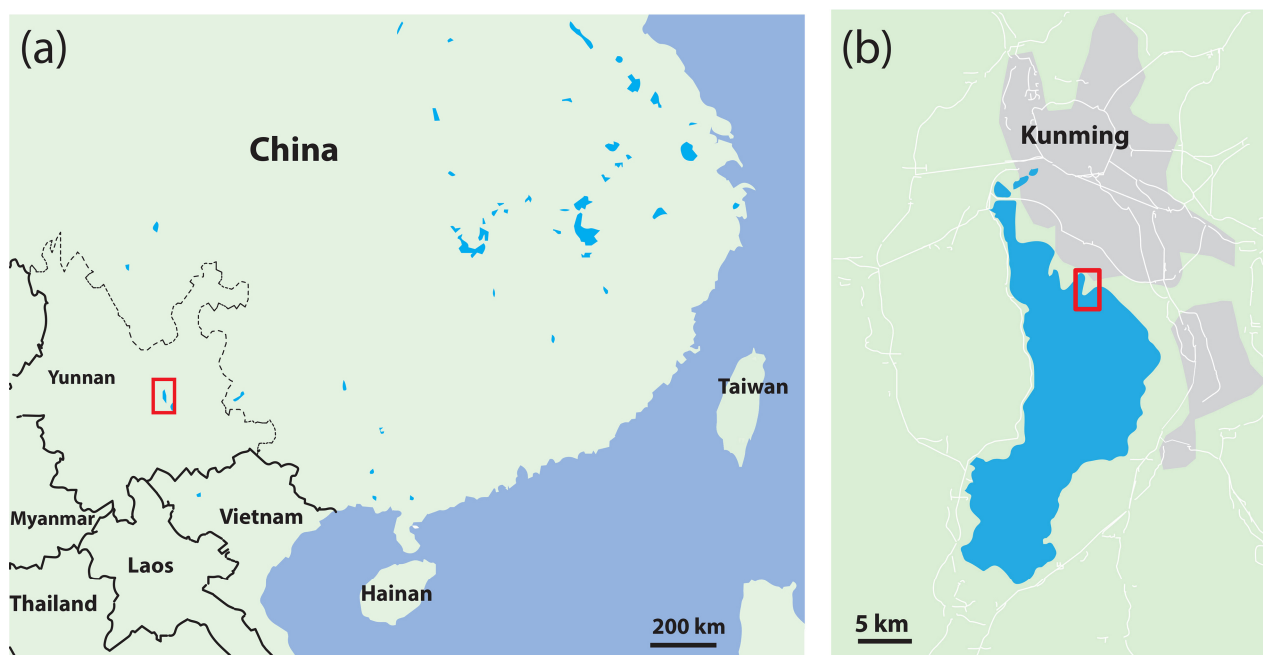


Figure 1. The sampling location of African clawed frogs, *Xenopus laevis*, on the northern shores of Lake Kunming, Yunnan Province, China. (a) The location of Kunming in Yunnan Province (dotted line), southern China, and (b) site of *X. laevis* on a peninsula in Dian Lake (blue) with major roads (white), and urban areas (grey).

scientific investigations, but most recently in the pet trade (Gurdon and Hopwood 2003; Van Sittert and Measey 2016; Measey 2017). The pet trade has been particularly important as an invasion pathway for amphibians (Kraus 2009), with the origin of propagules no longer being wild populations, but domesticated stock (Herrel and van der Meijden 2014) whose selective breeding may consequently increase the possibility of producing invasions.

Despite *X. laevis* having a native southern African distribution, the origin of nearly all African clawed frogs imported into the USA as pets is China (Measey 2017). As many as 50 000 individuals were imported in 2015, a number that has grown steadily over time (Herrel and van der Meijden 2014; Measey 2017). Exports from China appear to be entirely of an albino form of *X. laevis* (J. Rowley, *pers. comm.*; Kolby et al. 2014; Measey 2017). A recent review of invasive populations reported that African clawed frogs were present on four continents (Measey et al. 2012). Since then, new populations have been reported from Baja California, Mexico (Peralta-García et al. 2014), Florida USA (Hill et al. 2017) and more populations are present but are yet to be formally reported. All known invasions of *X. laevis* are of the wild type: individuals with heavy dorsal pigmentation with camouflaged patterns. It is questionable whether albino forms, most prevalent in the pet trade, are capable of establishing invasive populations as their coloration may increase their vulnerability to predation by birds and other visually orientated predators.

In this communication, we report on a new invasive population of albino African clawed frogs from Kunming Lake, Yunnan Province, mainland China (Figure 1). In 2014, one of us (SW) spotted a single adult



Figure 2. (a) A simple funnel trap made from a bucket placed in the cultural gardens adjacent to Kunming Lake (see Figure 1). (b) Placement of traps alongside the access road. (c) A specimen caught in the trap: Note the pink colouration of individuals signifies that they are all of albino form. (d) Specimens accessioned to the Institute of Zoology, Chinese Academy of Sciences, Beijing.

albino *X. laevis* swimming in an area inundated for aquaculture immediately adjacent to the northern shores of Lake Kunming, while carrying out surveys on invasive *L. catesbeianus* (see Wang et al. 2014). The individual was not captured, and no more were seen during subsequent visits. It was presumed that this was a single escaped pet (Blackburn et al. 2011; level C1 - survival), and not an established population (C2 reproduction). No reports were made at the time.

Materials and methods

On 22nd June 2018 (at 21h30), we set five simple funnel traps made from buckets, and baited with chicken livers (De Villiers and Measey 2017). These were placed at 10 m intervals along the shore of an aquaculture pond, immediately adjacent to the northern shore of Lake Kunming (24°55'26.0"N; 102°43'23.2"E; see Figure 2). Spotlighting into the water during placement of the traps did not reveal any *X. laevis*, but we did hear several *L. catesbeianus* calling throughout the time we were there. We returned to the traps at 06h45 the next morning and found five juvenile

X. laevis, all of the albino form. We also captured several loach, which are used by the local people for food. These ponds are for ornamental purposes, although local people do utilise them to stock and harvest fish, but no other amphibian adults or tadpoles. The specimens are deposited in the collection of the Beijing Academy of Sciences. We extracted DNA using a procedure from Shine et al. (2016) from each of the specimens captured and sequenced these for 16S using the primers (16Sc-L and 16Sd-H) described by Furman et al. (2015). Sequences from this study are submitted to EMBL-EBI European Nucleotide Archive (study accession number PRJEB30999 <http://www.ebi.ac.uk/ena/data/view/PRJEB30999>). We aligned our sequences with the 16S alignment of sequences from De Busschere et al. (2016), which included invasive populations from France, Italy and Portugal, as well as native range data from Furman et al. (2015). We also swabbed each individual for chytrid (*Batrachochytrium dendrobatidis* Berger et al., 1998) following Hyatt et al. (2007) and Wang et al. (2017a), and analysed the five samples obtained using qPCR assays (Boyle et al. 2004) with a Roche Light Cycler 480II Real-Time PCR system.

Results and discussion

Our sequences demonstrate that the established population of African clawed frogs near Kunming is from the same mitochondrial 16S haplotype 2 of De Busschere et al. (2016), as found in invasive populations in Portugal, Italy and part of the mixture of invasions in France. This clade corresponds with native populations in the far southwest of South Africa, including Jonkershoek and surrounding areas, which corresponds with exports from that area from the 1930s onwards (Van Sittert and Measey 2016). Our qPCR assays were all negative suggesting that these animals were not carriers of chytrid. This is significant as previous workers have disagreed about the potential impact of these invasive populations with respect to their chytrid status (Kraus 2015; Kumschick et al. 2017a, b), and this can significantly impact even objective risk assessment exercises (Kumschick et al. 2017a). It is important to note that samples from the population of American bullfrogs from this same site were also negative for chytrid (SW, *unpublished data*).

The establishment of an alien population of African clawed frogs on the Chinese mainland is surprising as Kunming, and most of China, does not fall into the predicted climate regime for this species (Ihlow et al. 2016; Measey et al. 2012), suggesting that this is another shift from the native niche (*cf* Rödder et al. 2017). China already has many populations of invasive amphibians including: American bullfrog (*L. catesbeiana* also heard during this study) and black-spotted frog, *Pelophylax nigromaculatus* (Hallowell, 1861) (Wang et al. 2016, 2017b), but it may be that these are currently under reported (van Wilgen et al. 2018). Pathways to these

invasions have come through the trade in live animals bred in the aquaculture industry for food, but also through Buddhist release ceremonies where animals are purchased from local markets and released into the environment (Liu et al. 2012). We do not know the specific pathway for this new population of *X. laevis* in China, but this species is not known from the restaurant industry in China, however it is associated with Buddhist release, and available as pets or pet food. Given the rural nature of this site away from major inhabited areas, we suggest that deliberate Buddhist release is the most likely pathway. If true, there may be records of when and where the release took place from local Buddhist communities (see Liu et al. 2012).

Although China is known to breed large quantities of *X. laevis* for export to the USA (Measey 2017), it is known that these animals are also available for purchase as pets within mainland China (Bai et al. 2012), and individuals are available to order through the internet (SW, *pers. obs.*). They have been previously collected from a pet shop in Kunming (Bai et al. 2012), but when we searched pet shops (including the one sampled in Bai et al. 2012) and markets in June and July 2018, we failed to find any *X. laevis* being sold.

That a population of albino African clawed frogs is established is of significance in that most of the propagules circulating the world are of this albino form (Measey 2017). Albino African clawed frogs are not thought to be able to revert to wild type, due to the lack of a wild type gene in these double recessives without a spontaneous mutation (B. Evans, *pers. comm.*). The ability of *Xenopus laevis* to survive in muddy opaque waters, such as the one in which we caught these animals, may allow individuals to escape predation pressure that would otherwise impede their establishment.

Kunming Lake represents a potential area of rapid expansion for this established population to invade a far greater area of China. Although *Xenopus laevis* is known to be able to move over land (Measey 2016; De Villiers and Measey 2017), use of irrigation channels (Fouquet and Measey 2006; Lobos and Measey 2002) and rivers (Measey and Tinsley 1998) appears to allow them to disperse more rapidly across the landscape. A large water-body the size of Dian Lake (298 km²) may be more difficult for multiple individuals to move and establish in a new site, due to increased numbers of predators (see information on vulnerability to predation above). We suggest that areas of aquaculture like the one in which we found this population are sampled for presence of *X. laevis* eDNA to discover whether an invasion has already taken place. In addition, we suggest sampling all known Buddhist release sites in this area (from Liu et al. 2012) using eDNA methodologies, to determine whether this is the most likely pathway for the release of this species. It would be desirable to know the environmental and economic impacts of this species in China, and compare this to information already published (Kumschick et al. 2017b; Measey et al.

2016). Of particular interest is the interaction between *L. catesbeianus* and *X. laevis*, which occur together at this site, and potentially in other areas. There is a potential for facilitation (invasion meltdown) through the removal of larval predators (such as odonate larvae; see Vogt et al. 2017) of *L. catesbeianus* by adult *X. laevis*, as seen previously between blue gill sunfish and American bullfrogs in Oregon, USA (Adams et al. 2003; Liu et al. 2018). Alternatively, adults of American bullfrogs and African clawed frogs are known as voracious predators of amphibians, especially (but not exclusively) of adults and larvae, respectively (Wu et al. 2005; Vogt et al. 2017; Measey et al. 2015; Courant et al. 2017), and this may reduce or augment the overall abundance of *L. catesbeianus*. Lastly, we suggest expanding the search for established and invasive populations of *X. laevis* in China. Given the known Major impacts of this species (Kumschick et al. 2017b), and the difficulty of determining presence of invasive populations, we suggest starting the search around known breeding facilities responsible for supplying the trade to the US exported from Hong Kong.

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